

CYTOLOGICAL STUDIES ON *SPIRONEMA*  
*FRAGRANS* LINDL. AND CERTAIN  
OTHER COMMELINACEAE



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INTRODUCTION

**S**PIRALIZATION and the behavior of chromosomes in *Tradescantia* have been studied in considerable detail by many cytologists (see Kuwada, 1932, 1938; Nebel, 1932a, 1932b, 1933, 1935; Kuwada and Nakamura, 1933, 1934a, 1934b, 1935; Sax and Humphrey, 1934; Nebel and Ruttle, 1935, 1936a, 1936b). *Spironema* and others of the Commelinaceae, however, seem to have been somewhat neglected, nor has the nucleolus of the Commelinaceae been much discussed. The present study deals fully with the structure and behavior of the mitotic and meiotic chromosomes of *Spironema fragrans* Lindl., reports the origin and the development of the nucleolus, and analyzes the relationship between the chromosomes and the nucleolus.

This study, undertaken in the Department of Botany, University of Michigan, was conducted under the direction of Professor Wm. Randolph Taylor, to whom the writer is greatly indebted for valuable suggestions and helpful criticism. She also wishes to express her appreciation to Dr. J. T. Baldwin, Jr., for assisting in revision of the manuscript.

MATERIALS AND METHODS

*Spironema fragrans* Lindl. was chosen for detailed analysis because of its large chromosomes and its many rapidly growing roots. *Neodonnellia grandiflora* Rose and *Rhoeo discolor* Hance served only

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root-tip smear method of Brown (1937), and the modification of LaCour's root-tip smear method for the Feulgen "nuclear reaction" by Mensinkai (1939a) were tried. All yielded satisfactory results, but the last two were superior, for they gave sharper differentiation of the chromatic elements. Because of difficulty in obtaining many polar views of metaphase plates in smears, the present study is almost exclusively based on preparations made by the paraffin method; smears were used only for selecting material.

It was not easy to secure adequate fixation, especially for the chromosomes between late prophase and anaphase. A number of fluids were employed: Bouin's, LaCour's 2BE mixture, Navashin's, Flemming's strong solution, Hermann's, and Taylor's modification of Flemming's fluid. In general the last three gave the best results. Addition of maltose or urea produced little improvement. The writer modified the Flemming type of fixative by mixing equal parts of 1 per cent chromic acid and glacial acetic acid, to 5 c.c. of which were added 2 c.c. of 2 per cent osmic acid in 1 per cent chromic acid. The spiral nature of the chromosomes was exhibited after two hours' fixation with this fluid. None of the fluids mentioned, however, revealed clearly the internal structure of mitotic chromosomes at metaphase and anaphase. Pretreatment by exposure of root tips to vapors of concentrated HCl or HNO<sub>3</sub> was advantageous for showing this internal structure. Proper duration of exposure was determined by experiment. With HCl, exposures producing equally good results varied from 30 seconds to 2 minutes; after an exposure of 5 minutes no clear figures could be found. HNO<sub>3</sub> required 15 minutes. The root tips were washed in distilled water for 30 minutes after the exposure, then fixed with one of the fluids mentioned above. Fixation was followed by washing and dehydrating; embedding in paraffin was done in the usual fashion.

Sections were cut from 5 to 12  $\mu$  in thickness and stained with iron-alum-hematoxylin. Newton's gentian-violet method and Smith's modification of this technique gave good results, too, but neither was found to be as practical as hematoxylin. The Feulgen stain, prepared after Coleman (1938), who used Norit as a decolorizing agent, was also employed. Sections treated in this way afforded excellent differentiation of chromosomes against a colorless background. The solution showed no change even after three months. Light Green was occasionally used as a counterstain.



For the study of meiotic chromosomes the smear method of Taylor (1924), in conjunction with the fixing fluids mentioned above, was employed exclusively. It proved to be the most satisfactory, although the author's modification of Flemming's method was better for demonstrating the doubly coiled nature of the chromosomes.

## OBSERVATIONS

### I. SOMATIC DIVISIONS

#### A. Number and Morphology of the Chromosomes

1. *Spironema fragrans* Lindl. — Darlington (1929), in a study of *Tradescantia*, described the chromosome morphology of *Spironema fragrans* as: two pairs of long chromosomes each with a submedian constriction, one pair with two submedian constrictions, and three pairs with subterminal constrictions, the shortest pair having satellites. Anderson and Sax (1936) reported in microspore nuclei of this species two chromosomes with median or submedian constrictions and four with subterminal constrictions. In the present material there are six pairs of chromosomes in root tips. Chromosomes *aa*, *bb*, and *c<sub>1</sub>c<sub>2</sub>* are large, with submedian constrictions. No satellites or secondary constrictions were observed (Pl. I, Figs. 1-5). The *a* and *b* pairs correspond to the two pairs of long chromosomes in Darlington's description. The *c* pair are heteromorphic and correspond to Darlington's third pair of chromosomes, but here no secondary constrictions were observed. One of the *c* pair of chromosomes, *c<sub>1</sub>*, has a constriction about one third of the way from one end (Pl. I, Figs. 1-4). This chromosome is more or less ring-shaped at metaphase. At anaphase its two arms straighten out, but the longer arm still bends slightly inward (Pl. I, Fig. 4). The *c<sub>2</sub>* chromosome is smaller than *c<sub>1</sub>* and has a submedian attachment. Its two arms are parallel to each other at early metaphase (Pl. I, Fig. 3) and form a V-shaped chromosome at anaphase, with one arm slightly shorter than the other (Pl. I, Fig. 4).

Richardson (1934) reported that in one slide of *Spironema fragrans* an extra fragment was found to lie sometimes very close to one of the submedian bivalents. Darlington (1929), Koller (1932), and Whitaker (1936) have observed fragments and unequal pairs of chromosomes in *Tradescantia*. Darlington attributed the inequality of these chromosomes to a loss through fragmentation. In the

present material there is no direct evidence for fragmentation except in one instance: in a preparation pretreated with HCl chromosome  $c_1$  shows a secondary constriction in its longer arm (Pl. V, Fig. 33a). If dimorphism of the  $c$  pair of chromosomes was caused by a fragmentation in chromosome  $c_2$ , this fragmentation might be expected to have occurred in the region of the secondary constriction. Such a secondary constriction was not observed in ordinary preparations, however. The abnormality may represent a hybrid character.

The  $dd$ ,  $ee$ , and  $ff$  pairs are short chromosomes with closely subterminal constrictions (Pl. I, Figs. 1-4). It is difficult to distinguish among them by size or morphology. Careful study shows that one  $d$  chromosome bears a single satellite at the distal end of the short arm, but this SAT chromosome is not much shorter than the other subterminal chromosome, as was true of the SAT chromosome described by Darlington. The other  $d$  chromosome has two satellites in tandem. The distal satellite is usually larger than the proximal one (Pl. I, Figs. 1, 6, 8); occasionally they are equal in size (Pl. I, Fig. 7) or the proximal one is larger than the distal (Pl. I, Fig. 9). If the interpretation of Mensinkai (1939b), that the SAT filament is a continuation of the chromonema and that the satellite is a spiralized end of the chromonema, is valid, it can be applied to explain the difference in size of the tandem satellites as due to the fact that the gyres contract differently in fixation. At anaphase, however, the two daughter chromosomes of the tandem SAT chromosome often show the same difference in number of satellites as the  $d$  pair of chromosomes at metaphase (Pl. I, Figs. 8-9), i.e. one daughter chromosome bears one satellite and the other two, in tandem. This suggests that one of the satellites may not have split. The dissimilarity of the two members of the  $d$  pair of chromosomes seems to be another piece of evidence for the hybrid nature of the strain of *Spironema fragrans* used in the present work. Chromosomes heteromorphic with respect to the presence or the absence of the satellites or in regard to their size have also been observed in *Galanthus* (Satô, 1937b), in *Lobelia* (Okuno, 1937), in *Allium* and *Trillium* (Mensinkai, 1939a), and in *Crocus* (Pathak, 1940).

2. *Neodonnellia grandiflora* Rose. — This plant appears not to have been previously investigated. The chromosomes are comparatively small and numerous in the root tips:  $2n = 32$  (Pl. III, Figs. 14-15). Two pairs are slightly submedian in attachment, and the



rest are all subterminal and arbitrarily divisible into three classes according to size: three pairs of larger chromosomes, two pairs having terminal satellites on the short arms and the satellites of one pair being smaller than those of the other (Pl. II, Figs. 10-11); five pairs of medium-sized chromosomes, without satellites; and six pairs of small chromosomes, one pair with terminal satellites on the short arms. There are, however, no sharp distinctions between the successive classes. The gradation in size from the largest to the smallest chromosomes is apparent in Plate II, Figures 10-11.

3. *Rhoeo discolor* Hance. — According to Darlington (1929), in the somatic division of *Rhoeo discolor* there are four chromosomes with the short arm less than half the length of the longer one and eight chromosomes submedian in attachment. Sax (1935), in a study of the meiotic chromosomes of *R. discolor*, stated that six of the twelve chromosomes in the ring are distinctly heterobrachial, but Anderson and Sax (1936) described, in the microspore nuclei, two chromosomes with subterminal fiber-attachment constrictions and four with median ones. In the present material three pairs of the chromosomes have more or less median fiber-attachment constrictions, and the other three pairs have the fiber-attachment region at about one third of the distance from one end, with one pair of this latter group bearing satellites (Pl. II, Figs. 12-13; Pl. III, Fig. 17). Darlington observed that the two SAT chromosomes are dissimilar and that one chromosome has a second constriction, but this is not true of the material at hand. The only dissimilarity observed by the writer is that often one of the medianly constricted chromosomes is somewhat ring-shaped at metaphase; no difference in size or in structure was seen (Pl. III, Fig. 17). The satellites were not clearly observed in anaphase (Pl. III, Fig. 18).

#### B. Chromosome Structure and Behavior in *Spironema fragrans*

1. *Prophases*. — In early prophase more or less regularly coiled threads evenly distributed throughout the nucleus are observed. In most instances the spirals appear as single coils (Pl. IV, Fig. 22), but occasionally, in the same nucleus, their duality can be seen in certain parts (Pl. IV, Fig. 23). It later becomes clearly evident that the spirals are double; they are in reality the sister chromatids formed at the last premetaphase. Still later the nuclei become much larger; the nucleoli are near the nuclear center instead of being in

the region of the poles, as at telophase; and anastomoses between paired chromatids occur less frequently, if at all. The sister chromatids are oriented as at telophase (Pl. IV, Fig. 24). The matrical material is not evident at this stage, the suggestion being that it contributes to the formation of the nucleoli. The coils of the chromatids now stretch considerably, and the double spirals are more clearly and regularly apparent (Pl. IV, Fig. 25). Next the sister chromatids gradually draw close together (Pl. IV, Fig. 26), this approximation being accompanied by a general contraction of the chromosome. An untwisting and a relaxing of the coils are also noticeable at this stage (Pl. IV, Fig. 27). Contraction with relaxation of the old spirals is caused by new spirals within them, which bring about an internal twist of the chromatid; the new spirals are not visible until late prophase. They may be called "minor spirals," corresponding to the minor spirals observed in meiosis. As contraction continues the chromosomes become thicker, and each chromatid assumes a corrugated appearance, which is the first sign of the minor spirals. Meanwhile the chromatids have untwisted rapidly. One can find (Pl. IV, Fig. 29) a longitudinal splitting throughout each chromatid of one chromosome, while the chromatids of another chromosome exhibit only an untwisting in certain parts. In later stages, seldom in early ones, a differentiation of the chromonemata and the achromatic matrix is seen. The matrical material gradually increases and, when stained, obscures the duality of the chromatids.

2. *Metaphase*. — As the nuclear membrane disappears, the individuality of each chromosome is quite apparent, and the chromosomes become aligned at the equatorial plate, their fiber-attachment regions being toward the center of the plate and their arms in the axis of the spindle. Relaxation of the relic spirals has been completed, but relational coiling can still be observed at early metaphase (Pl. V, Fig. 32). In preparations pretreated with HCl or HNO<sub>3</sub> the duality of the chromatids can be clearly seen. Each consists of two chromonemata; the metaphase chromosomes are quadripartite (Pl. IV, Fig. 30).

Relaxation of relational coils seems caused by the formation of minor spirals. In certain parts of the chromosomes, where minor spirals are observed, there are few relational coils; in other parts, where no minor spirals appear, more relational twists remain (Pl. IV, Fig. 29). This relaxation is completed by the time the minor spirals



reach their full expression. The untwisting and the development of the minor coils proceed from the ends of the chromatids toward the primary constriction, as is clearly shown in Plate IV, Figure 29.

3. *Anaphases*. — Each anaphase chromosome consists of two sister chromatids intertwined (Pl. IV, Fig. 31; Pl. V, Fig. 33). At this stage the satellites and their filaments are single (Pl. V, Fig. 33b). The matrix can be seen clearly at both metaphase and anaphase, but it is usually not uniformly distributed, probably because of variations in the effectiveness of the pretreatment.

4. *Telophases*. — The chromosomes become massed together as they reach the poles. The chromosome structure, though obscured, is the same as at anaphase, as can be seen in the arms of the chromosomes which project from the mass. Soon the chromosomes begin to expand in their distal parts, where they have the form of two intertwined spirals (Pl. V, Fig. 34). At this point, because of their expansion, the individuality of the chromosomes is again apparent, and — in spite of a decrease in stainability — their spiral nature is clearly revealed (Pl. V, Fig. 35). The karyolymph is differentiated at this stage, and a nuclear membrane is formed about each daughter chromosome group. Nucleoli are developed at the proximal ends of certain chromosomes. Anastomoses appear between the chromosomes. One has the impression that when the chromosomes draw apart from each other after being once closely appressed, interconnections remain. In certain parts of the chromosomes there is a single helical spiral; in other parts double spirals are intertwined (Pl. V, Figs. 35, 39). The chromosomes are prominently oriented at telophase (Pl. V, Figs. 36–39), a condition which results from the anaphasic arrangement of fiber-attachment regions toward the poles when the arms of the chromosomes are spreading behind. This disposition of the chromosomes is kept until prophase.

5. *Interphase*. — Interphase is characterized by a so-called “reticular” aspect arising from a further expansion of the telophase chromosomes. As expansion continues the spirals are much loosened and the dyads become extremely thin. At points where anastomoses are drawn out and where two chromatids intertwine thickenings occur, so that the nucleus has a knotted aspect. Later these dyad threads get thicker, and the anastomoses are lost. This increase in thickness is probably accompanied by contraction of the chromosome as a whole, and the contraction brings the sister chromatids so

close together that they give the appearance of being single spirals, as they do at early prophase.

### C. *The Nucleolus*

Van Camp (1924) considered that the nucleolus arises in telophase from small granules which flow together. Marshak (1931) described a close relationship between the chromosome matrix and the nucleolus substance. Gates and Pathak (1939) also observed the nucleolus to originate, in *Crocus*, as small globules, but at a particular region of definite chromosomes. Recent investigators (Nandi, 1937; Satô, 1939; Jacob, 1940; and Pathak, 1940) have reported small granules which are present in early telophase chromosomes and which contribute to the formation of the nucleolus. These workers, in contradiction to van Camp and Marshak, consider that the small granules aggregate in nucleoli-organizing regions of the SAT chromosomes and form a definite number of nucleoli under the influence of these regions, as described by McClintock (1934).

Heitz (1931a) postulated that all plants have satellites or secondary constrictions from which nucleoli arise. The nucleoli are correlated with the satellites in number, size, and position. Heitz (1931b) found, however, in *Vicia faba* and *V. monanthos*, which have only two SAT chromosomes, instances in which a micronucleus, formed from a single lagging chromosome without a satellite, had a nucleolus. This implies that even when the SAT chromosomes are absent nucleoli will arise. Matsuura (1938) observed that, whereas in *Paris hexaphylla* the nucleolar development is related to the SAT chromosomes, in *Trillium kamtschaticum*, where there are neither SAT chromosomes nor secondarily constricted chromosomes, the nucleolus nevertheless originates at given ends of certain chromosomes. He also reported, in an abnormal individual, that each chromosome formed a micronucleus and developed its own nucleolus. Satô (1936a, 1936b, 1937a, 1937b, 1938), basing his opinion on a series of investigations dealing with the relationship of the nucleolus and the SAT chromosome, agreed with Heitz's hypothesis. In 1938 he observed, however, that there are two SAT chromosomes in some species of *Leucojum* and *Sternbergia*, but four nucleoli in the same plants. All the evidence mentioned above shows a tendency to revision of the interpretation assigning the origin of the nucleolus to a particular chromosome pair and to a consideration of it as a func-



tion of the chromosomes as a whole. Since all these facts were observed in abnormal individuals, however, most investigators are still in agreement with Heitz's hypothesis.

1. *The relation of nucleolus and chromosomes.* — There is only one pair of SAT chromosomes in *Spironema fragrans*, but as many as three nucleoli are present at telophase (Pl. V, Fig. 38). At prophase and metaphase commonly one, very often two, occasionally three, nucleoli are to be seen (Pl. IV, Figs. 22, 24–27, 29). A striking fact is that, at metaphase, a nucleolus may be attached to a submedianly constricted chromosome without a satellite. Similar attachments were frequently found at later stages (Pl. VI, Fig. 45). The nucleolus is sometimes related to chromosome *a* (Pl. VI, Fig. 45), at other times to other chromosomes: to chromosome *b* (Pl. VI, Fig. 46) or chromosome *c*<sub>1</sub> or *c*<sub>2</sub> (Pl. VI, Fig. 47), for example. The nucleolus is not at a definite locus, though it is always attached to the longer arm of metaphasic chromosomes. A relation between the nucleolus and the subterminal chromosomes, including the SAT chromosomes, was observed in different nuclei (Pl. VI, Figs. 48–49). It seems that any chromosome may produce a nucleolus. Often a faintly stained membrane-like structure connects the nucleolus and the chromosomes (Pl. VI, Figs. 45, 47, 49). Though the nucleolus remains large and clear in metaphase, no nucleolar divisions have been found. The nucleolus usually disintegrates in the cytoplasm after detachment from the chromosomes.

2. *The origin and behavior of the nucleolus.* — Nucleoli usually do not appear before the daughter nuclei are well organized (Pl. V, Fig. 35). As telophase advances, a nuclear membrane arises, and the chromosomes become arranged at the periphery of the nucleus with the proximal ends of their arms still oriented at the pole. Soon a nucleolus appears in the midst of the chromosomes (Pl. V, Fig. 36). Its relation to the chromosomes can be seen from a polar view or, at least, from an oblique view (Pl. V, Figs. 36–38). One pair of SAT chromosomes is clearly attached to the nucleolus in the region of the satellites (Pl. V, Fig. 36). All nonsatellited chromosomes in the same nucleus are, however, also attached to the same nucleolus. Two comparatively large nucleoli and two small nucleolus-like granules are connected to the chromosomes with many faintly stained threads (Pl. V, Fig. 37). These two small granules are considered to be a beginning of nucleolar development; they probably fuse with each

other or with other collections of nucleolar material to form a larger nucleolus. There may be three nucleoli in the same nucleus (Pl. V, Fig. 38), and they may be in direct contact with the chromosomes or else connected with them by fine threads. These observations suggest that when the fiber-attachment regions of the chromosomes are regularly arranged and close together one large spherical nucleolus is formed. When they are loosely and irregularly arranged two or three nucleoli may be developed at the same time. Incipient nuclei usually show the same number of nucleoli (Pl. V, Fig. 39). The development of a nucleolus seems caused by the gradual addition of nucleolar material arising from the chromosomes (Pl. V, Fig. 36) or by mutual contact of small collections of nucleolar material (Pl. V, Fig. 37). If the chromosomes remain at the periphery of the nucleus the fully developed nucleoli become detached from them and move into the center of the nucleus. The nucleoli maintain the same position through interphase and early prophase (Pl. IV, Fig. 24).

At middle prophase, when the chromosomes become thicker, they usually surround the nucleolus (or nucleoli), and are so closely associated that it is difficult to detect which parts are in contact with it. This phenomenon is more prominent in *Rhoeo discolor* (Pl. IV, Fig. 28). These observations indicate a strong affinity between the chromosomes and the nucleolus at this stage; possibly there is then a transfer of material from the nucleolus. At later prophase only one or two chromosomes, and these not always the same ones, still remain in contact with the nucleolus. The relation of the nucleolus and the chromosomes at prophase is shown in Plate V, Figures 40-41, and Plate VI, Figures 42-44. Chromosomes with both submedian and subterminal constrictions and both satellited and nonsatellited chromosomes were observed to be attached to the same nucleolus (Pl. VI, Fig. 44), or one homologue was attached to the nucleolus and the other was near by (Pl. VI, Fig. 43, *right*), or two nonhomologous chromosomes were related to the same nucleolus (Pl. VI, Fig. 42, *right*). It is interesting to notice that, whatever the arrangement, the prophasic chromosome is always in contact with the nucleolus by the distal end of its short arm. The satellited chromosomes attach to the nucleolus by their satellites instead of by the SAT filament (Pl. VI, Fig. 43, *left*), as observed by Heitz (1931a) and many other investigators. Comparison of nucleolus-chromosome relationship at prophase with that at metaphase shows a movement



of the nucleolus from the distal ends of the short arms of the chromosomes to the distal ends of the long arms. When the nucleolus migrates from one end of a chromosome to the other, the nucleolar material may be evenly distributed through the whole length of the chromosomes. Apparently the membrane-like connection between the nucleolus and the chromosomes is material passing from the nucleolus to the chromosomes. Most investigators hold the view that the nucleolus remains firmly attached to its locus of organization, or near it, from the time the nucleolus is organized until its disappearance. Seemingly this is not true of the present material.

In *Rhoeo discolor* the origin and the behavior of the nucleolus are essentially the same as in *Spironema fragrans*. Here, too, there is only one pair of SAT chromosomes. The maximum number of nucleoli is four. The attachment of the nucleolus to each type of chromosome has been recognized in different nuclei at prophase. Three chromosomes with almost median constrictions are attached to nucleoli as shown in Plate VI, Figure 50. In the submedianly constricted chromosomes the nucleolus is usually attached at prophase to the distal end of the short arm, but occasionally to the long arm (Pl. VI, Figs. 51-52). At metaphase the nucleolus has been found only a few times (Pl. VII, Fig. 53). Movement of the nucleolus from one end of the chromosome to the other is not shown for this material, but in Plate VII, Figure 53, one can see the nucleolus attached near the middle part of the arm instead of at the end.

## II. MEIOTIC CHROMOSOME STRUCTURE AND BEHAVIOR IN *SPIRONEMA FRAGRANS*

### A. First Meiotic Division

The spiral structure of meiotic chromosomes, observed in 1880 by Baranetzky, has been described in many plants. Kuwada and other Japanese workers, in a series of papers, have described the chromonemata of metaphase chromosomes as "double-coiled." Similar reports have been made by Darlington (1935), for *Fritillaria*, Sax (1935), for *Rhoeo discolor*, Nebel and Ruttle (1936b), for *Tradescantia reflexa* and *Trillium erectum*, and Naithani (1937), for *Hya-cinthus orientalis*. Koshy (1934), Hoare (1934), Huskins and Smith (1935), Huskins (1937), and other workers, however, regard the chromonema at this stage as single-coiled.

1. *Diakinesis*. — In *Spironema*, though there are not sufficient preparations for studying earlier stages of meiosis, the double-coiled structure is apparently not present in the leptotene chromosome (Pl. VIII, Fig. 68). At diakinesis the chromosomes are so compactly twisted, and usually so deeply stained, that it is difficult to analyze the structure in detail (Pl. VII, Fig. 55). Because of the evidence of later stages the reticular appearance is considered to result from a number of spiraled chromonemata twisted together, and the less-stained substance is thought to be the matrix. The chromatids are mutually parallel, with corresponding gyres arranged at the same level, thus producing a regular reticulum. As the process advances, some of the chromosomes form bivalent rings (Pl. VII, Fig. 54a-c), and others have the two distal parts parallel (Pl. VII, Fig. 54d). In late diakinesis there are three more or less ring-shaped tetrads and three cross-formed associations (Pl. VII, Fig. 56). The so-called "kinetochores" or "centromeres" are clearly seen in Plate VII, Figure 56, a pair in each dyad. The transition from the stage in Figure 54 to that in Figure 56 must be rapid, for no intermediate forms were observed. The internal structure of the chromatids, at the stage shown in Figure 56, gives the appearance of transverse bands embedded in the matrix. Under certain conditions of distortion this structure — continuous spirals and longitudinal splitting of each of the chromatids — can be observed more clearly (Pl. VIII, Fig. 62).

2. *Metaphase*. — Some of the chromosomes begin anaphasic movement before the others (Pl. VII, Fig. 57), and it is difficult to find a complete chromosome set of three rings and three crosses at full metaphase. However, three ring-shaped chromosomes from one cell (Pl. VIII, Fig. 63) and three cross-shaped chromosomes from another are illustrated (Pl. VIII, Fig. 64). Sax and Humphrey (1934) observed, in *Tradescantia*, that during metaphase the coiled chromatids of each chromosome gradually separate until each homologue consists of two separately coiled threads. In the present material, not only do sister chromatids lie parallel in independent spirals, as described by Kuwada (1938) in *Tradescantia reflexa*, but sister chromonemata are also side by side before metaphase. The four chromonemata of each dyad are so arranged that when viewed laterally they appear as two. When there is a twisting, however, especially



at the ends of the chromosome arms, the four-strand condition can be clearly seen (Pl. VIII, Fig. 63a-b; Pl. III, Fig. 20a, f, h).

The metaphase chromosomes have major and minor spirals. The major coils gradually increase in diameter as karyokinesis proceeds. Coiling appears to be unidirectional on a given side of the spindle attachment. The minor spirals are too small to be studied in detail. As the two dyads of the rings gradually separate they disjoin first at the distal ends of the short arms and then at the distal ends of the longer arms (Pl. III, Fig. 20a-g). One ring-shaped tetrad has two constrictions, one apparently the fiber-attachment constriction, the other subterminal and usually disappearing in later stages (Pl. VII, Fig. 57). Six submedianly constricted dyad chromosomes are formed from the metaphasic ring tetrads (Pl. III, Fig. 19; Pl. VII, Fig. 57). The dyads from each cross-shaped tetrad are rodlike or have almost terminal constrictions (Pl. III, Fig. 20h-j). A side view of this stage is shown in Plate VII, Figure 58. The sister chromatids of each dyad separate distally but are still held together at the fiber-attachment region (Pl. VII, Fig. 59). Each half dyad consists of two double-coiled chromonemata.

3. *Anaphases*. — The anaphase chromosomes move into mirror-image positions at the poles (Pl. VII, Fig. 60). During anaphase eight chromonemata in each geminus become discernible (Pl. VIII, Fig. 65). The major coils are more definite: five in the long arms of the chromosomes with submedian constrictions and of those with subterminal ones; three in the short arms of the submedianly constricted chromosomes. Generally one or two chromosomes reach the poles before the others (Pl. VII, Fig. 60).

4. *Telophases and interphase*. — During telophase sister chromatids become parallel, and the four chromonemata interconnect; a reticulum arises; and the minor coils of the chromonemata gradually lose their identity (Pl. VIII, Fig. 66). Individual chromosomes now appear, very much as they did at diakinesis, the chromonemata being arranged in the same manner as they were then (Pl. VIII, Fig. 69). Later stages exhibit a looser reticulum, involving a multitude of apparently threadlike interconnections between chromosomes (Pl. VIII, Fig. 67); individual chromosomes remain recognizable. The chromosomes gradually become strongly stainable and compact masses (Pl. IX, Fig. 70).

B. *Second Meiotic Division*

1. *Prophases*. — As prophase of the second meiotic division begins, the chromosomes unravel from the compact masses; this process is accompanied by a progressive loss of stainability. They then expand, become more uniform in diameter, and finally spread evenly throughout the nucleus as loosely coiled threads (Pl. IX, Fig. 71). In later stages it is not impossible to trace each chromosome for its whole length (Pl. IX, Fig. 72), and all chromosomes in a single nucleus may be analyzed. The split chromosomes have threadlike connections at the fiber-attachment region; distal regions diverge. Chromosome structure and behavior here are much like those observed by Taylor (1931) in *Gasteria*. In the present material three chromosomes are submedian and three are subterminal, one of the latter bearing a pair of satellites. No satellites or subterminal fiber attachments were found in the chromosomes of the first meiotic division; they were probably obscured by the extreme thinness of the chromonemata and the abundant matrical substance.

Taylor (1931) found, in *Gasteria*, that a splitting takes place at late prophase of the second meiotic division. In the material studied by the writer the splitting of each of the chromatids was seen to take place at late diakinesis and was traced to the first telophase, where the two pairs of chromonemata in each dyad merge into a reticular structure. This four-strand condition reappears optically at late prophase of the second division (Pl. IX, Fig. 73). Sister chromonemata at this stage are closely twisted about each other instead of being mutually parallel, as they were in the first meiotic division.

According to Nebel (1932a), in *Tradescantia reflexa* four chromonemata are evidently dyad chromosomes from the first telophase through the second prophase, where two double spirals are formed for every four chromonemata. The gyres of the double spirals correspond initially to those of the first meiotic chromosomes, but soon the two chromonemata of the double spiral are thrown into secondary spirals, which are very small. This seems similar to the condition observed in second prophase of the present material, though here the formation of secondary spirals probably takes place earlier. They are considered to occur at the end of the first telophase, when the chromosomes draw together and form compact masses. As the chromosomes emerge from these masses the two chromonemata

of each half dyad must be so closely entwined that they appear single at early second prophase. When the chromosomes contract and thicken, the duality is brought out again. With the reappearance of the secondary spirals the old coils gradually straighten (Pl. IX, Fig. 73). Only a few satellites were found (Pl. VIII, Fig. 61a; Pl. IX, Fig. 72).

As the process advances, the chromosomes greatly shorten and thicken and become differentiated into their constituent matrix and chromonemata. The number of secondary spirals is much reduced (Pl. IX, Fig. 74). The half dyads may or may not separate before the nuclear membrane disappears.

2. *Metaphase*. — As the nuclear membrane vanishes the dyad chromosomes draw close together into apposition and shorten somewhat (Pl. III, Fig. 21; Pl. IX, Fig. 75). They lie around the edge of the plate with their fiber-attachment regions toward the center. Kuwada and Nakamura (1935), in *Tradescantia reflexa*, observed the chromonemata at this stage to be singly coiled and parallel to each other. In the present material, however, they are intertwined, as described by Taylor (1931), Nebel (1932a), and others. The chromosomes of this division are essentially like those of a somatic division, though shorter.

3. *Anaphases*. — Anaphasic movement is assumed to be completed quickly and the daughter chromosomes oriented at the poles (Pl. IX, Fig. 76). Two polar views of the anaphase group are shown in Plate III, Figure 16. The anaphase chromosomes are usually less thick than the metaphase ones, but structurally they are the same. If examined by ordinary methods the internal structure of chromosomes at second metaphase and second anaphase is obscure. Their coiled nature and the duality of each half dyad is revealed more clearly by preliminary natural desiccation. The double nature of half dyads at second anaphase has also been described by Taylor (1931), in *Gasteria*, Nebel (1932a), in *Tradescantia*, Sax (1935), in *Rhoeo*, and Kuwada and Nakamura (1935), in *Tradescantia*.

4. *Telophases*. — The twisted condition of the chromonemata is still visible at telophase (Pl. X, Fig. 77). The chromosome mass becomes enveloped by a new membrane, the nucleus enlarges, and the chromosomes lengthen greatly (Pl. X, Fig. 78). The duality of each chromosome becomes clearly evident. As the process goes on, the chromosome outline is gradually lost, and eventually the chro-



matic structure spreads uniformly throughout the nucleus (Pl. X, Figs. 79-80).

#### SUMMARY

1. *Spironema fragrans* shows six pairs of somatic chromosomes, of which three pairs are submedian and three pairs subterminal. One pair of the subterminal chromosomes bears satellites. Two pairs of the chromosomes are considered to be heteromorphic.

2. *Neodonnellia grandiflora*, with sixteen pairs of somatic chromosomes, shows two submedian pairs and fourteen subterminal pairs. Three pairs of the subterminal chromosomes bear satellites.

3. *Rhoeo discolor* appears to have six pairs of somatic chromosomes, with three pairs having an almost median fiber attachment and three pairs having a fiber attachment at one third of the distance from one end.

4. The somatic chromosomes in *Spironema fragrans* are double throughout mitosis and become quadruple in premetaphase. In meiosis the leptotene threads appear to be double. Division of the chromatids takes place in premetaphase of the first meiotic division. Each homologous chromosome at first metaphase consists of four chromonemata which appear as four independently double-coiled spirals. At anaphase each contains a pair of chromatids which part along their distal ends and hold together only at the fiber-attachment region. Each chromatid of the dyad consists of a pair of chromonemata which are parallel to each other, with their corresponding turns arranged at the same level.

5. At second meiotic division new spirals are formed which uncoil the relic spirals. The second metaphase and anaphase chromosomes are therefore composed of minor spirals only. The duality of the second anaphase and telophase chromosomes is clearly visible.

6. The behavior and the origin of the nucleoli in somatic telophase were studied in *Spironema fragrans*. The nucleolar material is considered to be collected at the fiber-attachment region of each chromosome, and the nucleoli were observed to be accumulated in the midst of, or in the spaces between, the centromeres of the chromosomes. The relation between nucleolus and chromosomes was traced at prophase and metaphase in *S. fragrans* and *Rhoeo discolor*. The nucleoli are not particularly attached to the SAT chromosomes or to any certain chromosomes. The chromosomes were seen to have equal

chances of forming nucleoli at telophase, and likewise to have an equal chance of being attached to the nucleolus at prophase and metaphase. A migration of the nucleolus from the distal end of the short arm of the chromosome to that of the longer arm, as the mitotic process advances from prophase to metaphase, was observed in *S. fragrans*. It is believed that the nucleolar substance contributes to the matrix of the chromosomes.

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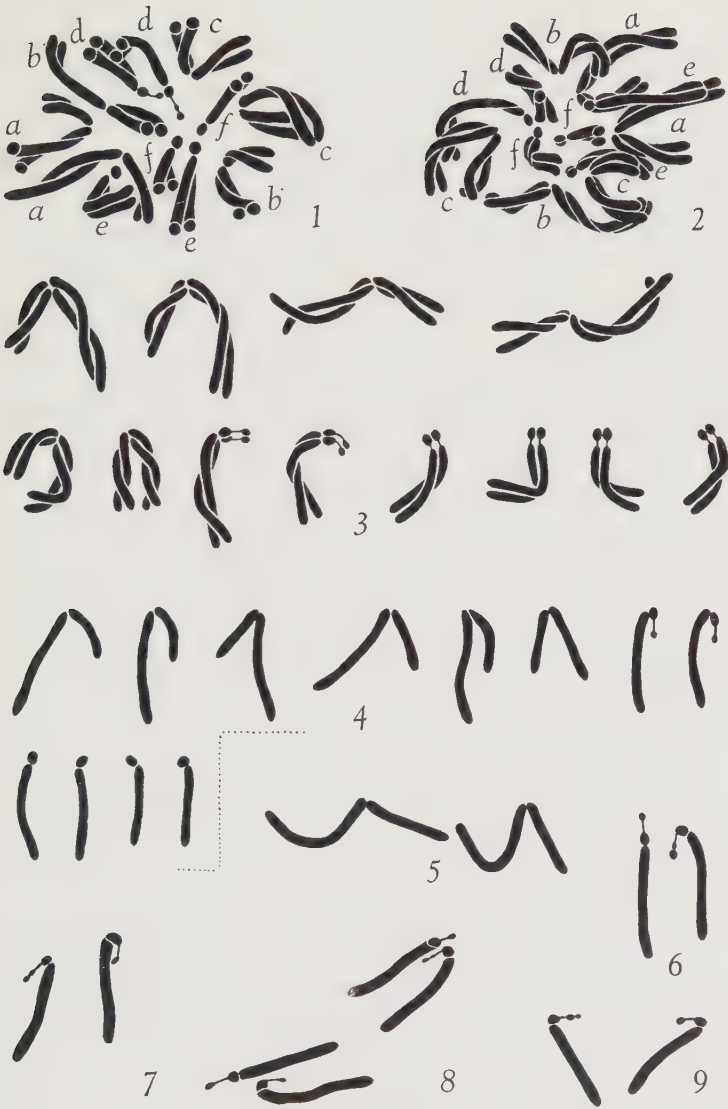
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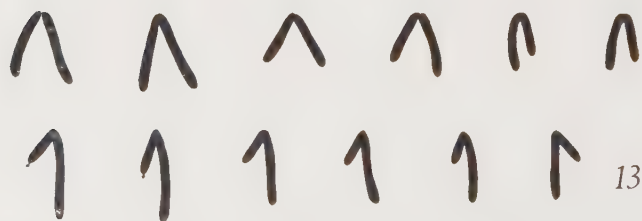
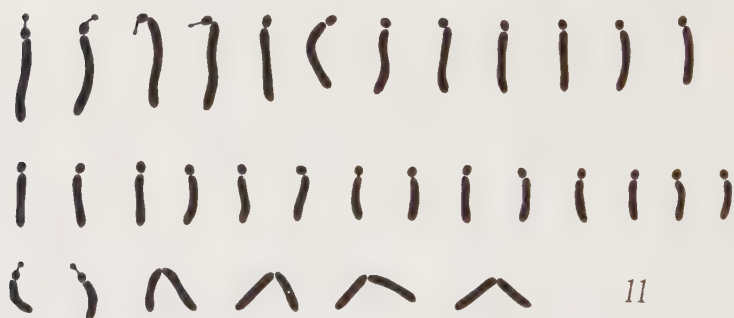
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PLATES I-X



FIGS. 1-9. Chromosomes of *Spironema fragrans*. × 2000





Figs. 10-13. Chromosomes of *Neodonnellia grandiflora* (Figs. 10-11) and *Rhoeo discolor* (Figs. 12-13).  $\times 2000$



FIGS. 14-21. Chromosomes of *Neodonnellia grandiflora* (Figs. 14-15), *Rhoeo discolor* (Figs. 17-18), and *Spironema fragrans* (Figs. 16, 19-21).  $\times 2000$  except Figure 17 ( $\times 1600$ )



FIGS. 22-31. Prophase chromosomes of *Spironema fragrans* (Figs. 22-27, 29-31) and *Rhoeo discolor* (Fig. 28).  $\times 2000$  except for Figures 30-31 ( $\times 2800$ )

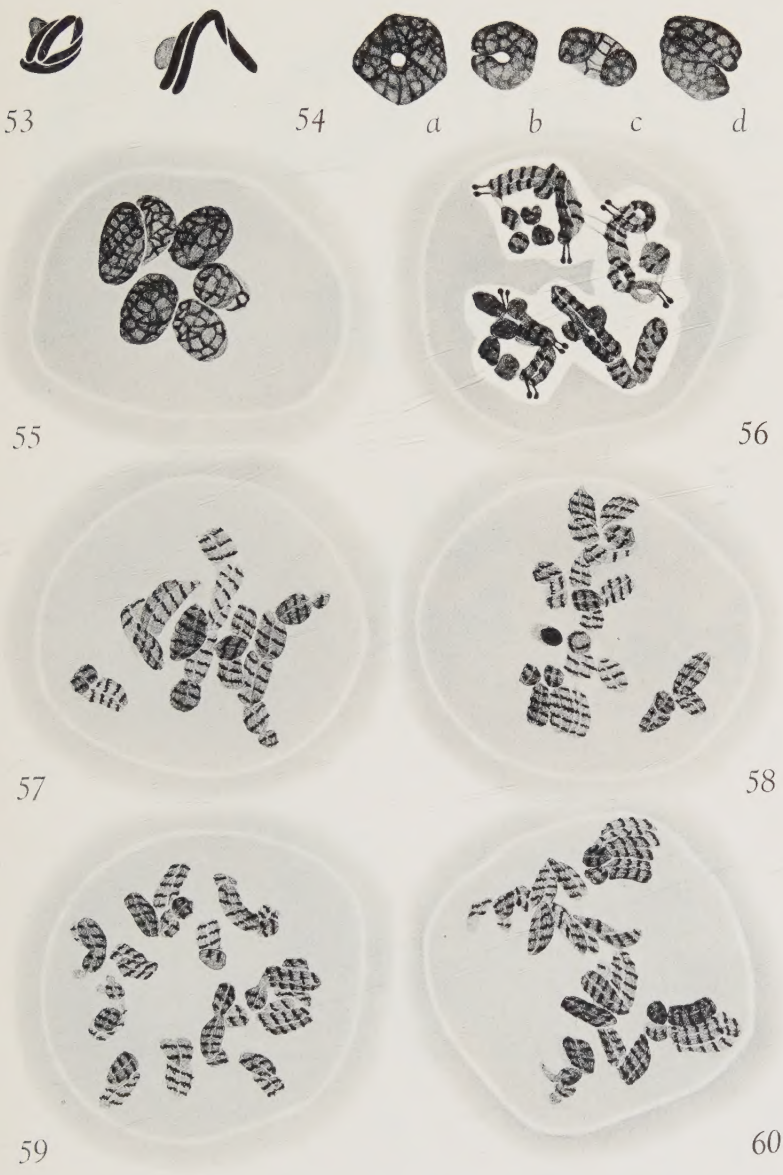




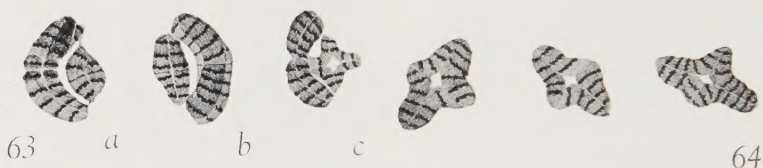
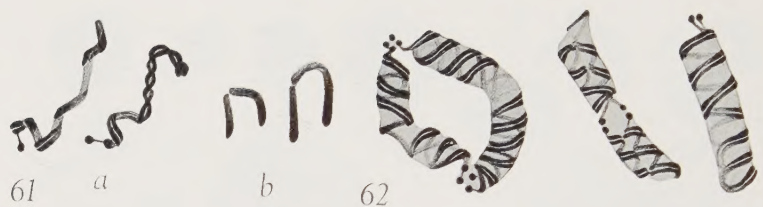
FIGS. 32-41. Chromosome development in *Spironema fragrans*. Figures 32, 34, 36, 40-41,  $\times 2000$ ; Figures 33, 37,  $\times 2800$ ; Figures 35, 38-39,  $\times 2400$



FIGS. 42-52. Nucleolus-chromosome relation in *Spironema fragrans* (Figs. 42-49) and *Rhoeo discolor* (Figs. 50-52).  $\times 2000$



FIGS. 53-60. Meiosis in *Rhoeo discolor* (Fig. 53) and *Spironema fragrans* (Figs. 54-60).  $\times 2000$

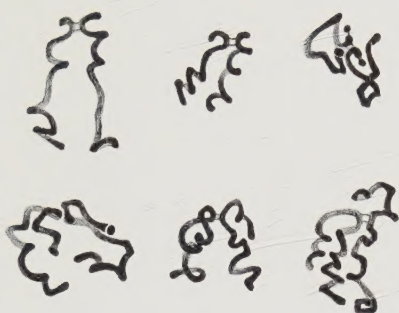


FIGS. 61-69. Meiosis in *Spironema fragrans*.  $\times 2000$

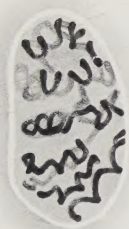




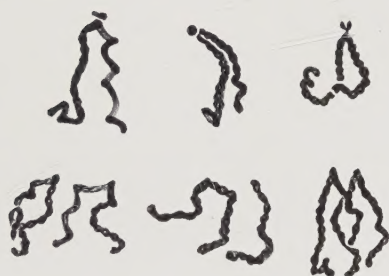
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FIGS. 70-76. Meiosis in *Spironema fragrans*.  $\times 2000$



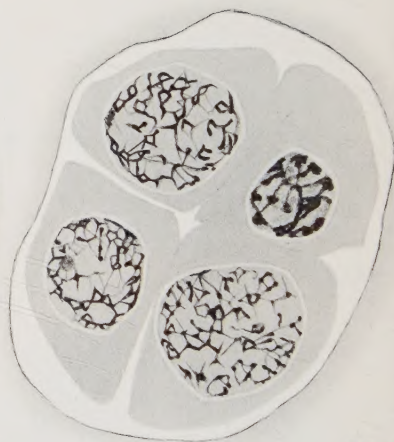
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FIGS. 77-80. Meiosis in *Spironema fragrans*.  $\times 2000$